Guidelines for the Blood Transfusion Services

Chapter 8: Evaluation of novel blood components, production processes and blood packs: generic protocols

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Chapter 8:

Evaluation of novel blood components, production processes and blood packs: generic protocols

8.1: Aims and introduction

This chapter aims to describe how a proposed novel blood component, production process or blood pack is to be evaluated to:

- gain sufficient data to validate the component and production method
- gain sufficient data to support the clinical use of the component
- allow the Standing Advisory Committee on Blood Components (SACBC) to recommend to the Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (JPAC) that the component should be included in the Red Book, either within the ‘Specifications for blood components‘ section or as a Provisional Component specification in the ‘Provisional Components’ section
- provide sufficient information to prevent all Blood Establishments (other than those performing a full evaluation) from having to complete a full validation of the novel component before it enters routine production. They will only need to undertake installation and process validation.

The chapter starts by identifying the steps that a group of investigators will need to undertake to submit a novel blood component for inclusion in the Red Book (see Table 8.1a), thereby allowing it to be produced on a routine basis throughout the UK. Guidance on assessing the degree of novelty of components prior to embarking on the process is given in Table 8.1b.

It is recognised that some novel components may be developed by a group of investigators in conjunction with a commercial company undertaking speculative research. As a result, the group of investigators may wish to enter the process at Step 8. In this case the SACBC will expect any requirements for data collection in the preceding steps to be complied with when the protocols and reports are submitted to the SACBC Chair for consideration. If sufficient data are not included then a request for extra data will be made (Step 9).

It is also recognised that there may be a need for Blood Establishments to produce blood components for clinical use on a temporary basis. This may be to undertake a clinical study or operational assessment of a new component in order to inform the decision as to whether there is a need to manufacture the component

on an ongoing basis. In such circumstances the most appropriate course of action is to seek approval for a Provisional Component specification (see section 8.1.1 and Table 8.1c).

Guidance on how specific novel components should be tested is included in sections 8.2–8.5, and is followed by information on generic protocols for the evaluation of apheresis equipment (section 8.6) and blood packs (section 8.7). For guidance on phases of validation and sample size, please refer to Table 8.1d

Table 8.1a Steps for evaluation of novel components

Step	Details	Information
1. Investigators identify requirement for a novel blood component.	The requirement must be derived from R&D work or as the result of clinical discussions. The blood component needs: • to fulfil an unmet clinical need OR • provide production benefit and have a Blood Service proposer. Investigators will need preliminary data to support their application.	The new component may be derived from a commercially available product. In this case data to support the submission may be derived from the manufacturer. Investigators must critically appraise data already available. All data must be maintained on file. Data will be used to demonstrate validation has been completed in support of Blood Establishment licensing activities. Data required may include clinical outcome.

2. Investigators may obtain initial advice from the SACBC Chair as to whether the component should be treated as novel. Table 8.1b describes likely degrees of novelty and clinical use of components.	Yes: Go to Step 3. No: Undertake local validation and produce the component locally under the general principles of good manufacturing practice and the Red Book (Phase 1, Table 8.1d).	The proposed new component may require evaluation even if it complies with existing Red Book guidelines if: • a new production technique is involved (e. g. leucocyte-depleted red cells produced by apheresis). • there are different steps in the production process (e.g. white-cell filtration immediately following collection). • definitive advice about the need for full-scale evaluation will be provided from the SACBC following a written submission.
Characterise the new blood component		
3. Investigators define the intended specification for the blood component.	Written specification to include: • expected characteristics (e.g. leucocyte count) • testing characteristics (blood grouping, microbiology etc.) • sampling time, sampling method and sample handling conditions to confirm that the component meets specification. Reference should also be made to the research papers from which the specification is derived.	Specify all key points which will allow subsequent production of the component to be well controlled.

4. Write the protocol for component evaluation (Phase 0).	Investigators' group writes procedures for: • component production • monitoring of performance • clinical use • outcome measurement • adverse incidents in production/use of the blood component or uses manufacturer's documentation to produce 'in-house' protocols.	Principles of good clinical and good manufacturing practice should apply. Comply with generic protocols (Table 8.1d and sections 8.2–8.7). Laboratory studies should comply with local standards. Must include in the procedure the sampling regimes, data analysis and expected ranges, which will be used to confirm that production of the component is under control. Must include detail of the data analysis methods.
5. Investigators should ensure their protocol complies with Chapter 8 and may seek advice from the SACBC.		
6. Obtain ethics committee approval, if required.		Must comply with local consenting and ethics policies for the use of donated material.
7. Investigators apply protocol.	Document evidence of protocol being implemented. Investigation should be subject to independent quality audit.	Audit may be carried out on behalf of collaborating manufacturers even though this may be confidential regarding the data collected. A summary outlining non-compliances against good clinical and manufacturing practice must be made available to the Blood Transfusion Service involved, for submission as part of the supporting documentation to the SACBC.
Obtain SACBC listing of the component		

8. Investigators submit report and supporting data and a draft Component specification to the SACBC for consideration.	Investigators review outcomes and produce a report, which summarises findings and supports the case for a new blood component to be listed. The SACBC decides if: • the blood component is novel • the data support the ability to produce the blood component on a regular basis • the blood component is efficacious and safe.	Investigators who have been conducting speculative research with a manufacturer may enter the process at this point. This may also include data supplied by manufacturers, other Blood Services, and published studies. Investigators should submit a draft specification for the component.
9. The SACBC decides whether the component may be recommended for inclusion in Chapter 7, the 'Specifications for blood components' section of the Red Book guidelines.	If the SACBC decides that the blood component will be listed, it submits this recommendation to the JPAC, providing copies of the data and report used to accept the new blood component. If the SACBC decides that the blood component will not be listed, it informs the submitting group and provides an explanation.	The SACBC may request further data in support of the submission prior to listing the blood component.
Joint Professional Advisory Committee		
10. Consider the recommendation that a new component should be listed.	Notify the SACBC of the decision. If not accepted, provide the SACBC with detailed reasons for the decision. If accepted, notify Medical Directors and Quality Managers of the four UK Blood Transfusion Services. Include the Component specification in Chapter 7 of the Red Book guidelines.	
SACBC		

11. Communicates the JPAC decision to appropriate parties.	If accepted inform investigators who must complete a Component Code Request form and submit to Chair of the SACBC. The form is assessed by the SACBC and if approved this request is passed to the SACIT to proceed with the provision of appropriate labels. If not accepted inform investigators, with supporting reasons.	Component Code Request form is available in the Document Library on the JPAC website (which also includes guidance on requesting codes for non-novel components).
SACIT		
12. Provides codes for the new blood component.	Code will be unique. ISBT 128/ABC Codabar will be supported.	
13. Provides a component label and updates the UKBTS Component Portfolio.	Label will be unique. Completed Component Code Request form returned to the SACBC and requestor.	Label text will describe the key attributes of the component.
Blood Establishment		
14. Begin production of the new blood component.	Base procedures on those used during validation studies. Complete installation and process validation (Phase 1, Table 8.1d).	Demonstrates without redoing the above validation that the blood component produced is equivalent to that defined in the UK guidelines.
15. Produce the blood component routinely.	Confirm procedures.	Continue to monitor production to the Red Book specification (Phase 2, Table 8.1d or routine quality monitoring).

Table 8.1b Degrees of novelty of blood components

Degree of novelty	Regulatory	Clinical data /experience	Extent of laboratory validation required	Clinical use

Very High	Produced using medical device /process that is **NOT** CE/UKCA/UKNI marked, or covered by manufacturer's IFU. A notice of no objection from the MHRA would be required for any trial.	No clinical use in humans	Extensive laboratory validation and data in relevant animal models. Likely to have to define all key critical variables that determine product quality.	First in man/phase I studies. HRA approval required and not to be used outside of approved study.
High	Produced using medical device that is **NOT** CE/UKCA /UKNI marked, or covered by manufacturer's IFU. A notice of no objection from the MHRA would be required for any trial.	Clinical data likely to be limited to small scale studies as part of R+D, or historical use or use outside of Europe.	Extensive laboratory validation. Likely to have to further define some critical variables in product quality.	Likely to be a phase II /III research study. HRA approval required and not to be used outside of approved study.
Medium	Produced using medical device that is CE/UKCA/UKNI marked, but **OUTSIDE** of its intended use or manufacturer's instructions for use (IFU). A notice of no objection from the MHRA would be required for any trial.	Clinical data likely to be limited to small scale studies as part of R+D or historical/small scale clinical use or use.	Laboratory validation required guided by data to date and intended use. Likely to have to validate changes to key variables such as temperature or duration of storage.	Likely to be a phase II /III research study. HRA approval required and not to be used outside of approved study.

Low	Produced using medical device that is CE/UKCA/UKNI marked **WITHIN** its intended use & manufacturer's IFU. Currently NO specification in Red Book or not for the usage proposed. Likely to be a specification for product elsewhere e. g. Council of Europe or AABB guidelines. Use would not be precluded by content of BSQR or relevant EU directives. Use would require local validation and approval by SACBC /JPAC.	Not used recently in UK, or change in clinical use of an existing component. Might be in routine use elsewhere internationally but not the UK.	Extent of laboratory work guided by nature of change to be made and any uncertainties in published data e.g shelf-life.	Use might either be considered a change in clinical practice or as part of an approved research study, to be determined based on clinical usage/data to date. Use might be restricted in first instance to pilot sites. Safety might be monitored through haemovigilance which might be enhanced above standard based on risk.
Standard component (therefore not a 'provisional component specification')	Produced using medical device that is CE/UKCA/UKNI marked **WITHIN** its intended use & manufacturer's IFU. Has **APPROVED** specification in Red Book. In routine use in the UK and manufactured to approved specification in Red Book.	Widespread clinical experience from routine use in the UK and elsewhere.	Introduction would require local validation.	As per clinical guidelines.

8.1.1: Provisional Component specification

This process should be used where it is uncertain whether there will be a requirement to produce the novel component on an ongoing basis, yet there is a need for clinical use of the component. Provisional Component specifications once approved will be posted in the 'Provisional Components' section of the Red

Book.

The purpose of approval of a provisional component specification is to:

- ensure that there is sufficient data to support progression from phase 0 to phase 1 & 2 studies and the clinical use of the component.
- document a draft specification for the component including suitable quality monitoring parameters and testing regime for phase 1 and 2 studies.

Table 8.1c Steps for approval of a provisional component specification

Step	Details	Information
Investigators undertake steps 1-7 in Table 8.1a.	Gather Phase 0 and other data necessary to proceed to step 8.	Seek advice from SACBC in advance with respect to validation requirements if needed.
Obtain SACBC listing of the provisonal component		
8. Investigators submit report and supporting data and a draft Provisional Component specification to the SACBC for consideration.	Investigators review outcomes and produce a report, which summarises findings and supports the case for a provisional component to be listed. The SACBC decides if: • the blood component is novel • the data support the ability to produce the blood component for its intended use • the blood component is efficacious and safe. • the specification and associated quality monitoring for subsequent phases of study are adequate • data support progression to phase 1 & 2 studies and clinical issue of the component.	Investigators who have been conducting speculative research with a manufacturer may enter the process at this point. This may also include data supplied by manufacturers, other Blood Services and published studies. Investigators should submit a draft specification for the component.
9. The SACBC decides whether the component may be recommended for inclusion in the 'Provisional Component' section of the Red Book guidelines.	If the SACBC decides that the blood component will be listed, it submits this recommendation to the JPAC, providing copies of the data and report used to accept the new blood component. If the SACBC decides that the blood component will not be listed, it informs the submitting group and provides an explanation.	The SACBC may request further data in support of the submission prior to listing the blood component.
Joint Professional Advisory Committee		

10. Consider the recommendation that a new component should be listed.	Notify the SACBC of the decision. If not accepted, provide the SACBC with detailed reasons for the decision. If accepted, notify Medical Directors and Quality Managers of the four UK Blood Transfusion Services. Include the Component Specification in the 'Provisional Components' section of the Red Book guidelines.	
SACBC		
11. Communicates the JPAC decision to appropriate parties.	If accepted inform investigators who must complete a Component Code Request form and submit to Chair of the SACBC. The form is assessed by the SACBC and if approved this request is passed to the SACIT to proceed with the provision of appropriate labels and component code. If not accepted inform investigators, with supporting reasons.	Component Code Request form is available in the Document Library on the JPAC website (which also includes guidance on requesting codes for non-novel components).
SACIT		
12. Provides codes for the new blood component.	Code will be unique. ISBT 128/ABC Codabar will be supported.	
13. Provides a component label and updates the UKBTS Component Portfolio.	Label will be unique. Completed Component Code Request form returned to SACBC and requestor.	Label text will describe the key attributes of the component.
Blood Establishment		
14. Proceed to phase 1 & 2 studies.	Base procedures on those used during validation studies. Complete installation and process validation.	Monitor component against trial specification.
15. Decide if there is a need to produce the component on an on-going basis.		Submit report on phase 1 and 2 studies and clinical data if relevant to SACBC and the final specification for the component to be included in the Red Book.

Table 8.1d Summary of testing numbers required for evaluations and validations

Process	Testing	Phase 0	Phase1	Phase 2 (see 8.7)	Local process validation
Whole blood collections	Component evaluation	10-16 See Tables 8.2 to 8.5	None	None	None
	Quality monitoring	10-16 100% tested	125 100% tested	2000 from each of two batches Minimum 1% tested or as determined by statistical process control	125 100% tested
Apheresis collection	Component evaluation	10–16 See Tables 8.2 to 8.5	None	None	None
	Quality nonitoring	10–16 100% tested	125 100% tested	300 100% tested	10 (each machine) 100% tested

8.2: Evaluation of new red cell components for transfusion

8.2.1: Introduction

In establishing any novel component, the development process is expected to involve three stages (see Table 8.1d):

- **Investigation (Phase 0):** Initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10 -16) to establish concepts. This should involve *in vitro* studies with serial sampling, and may also involve *in vivo* studies. Components produced during this phase should not be used for transfusion. For clarity the guidance on which tests need to be performed is as shown in Table 8.2.

- **Validation (Phase 1/Phase 2):** Operational validation on a larger number of units (e.g. 125) to establish routine operation of the technique, normally testing for those parameters listed in the current edition of the Red Book. These tests may be supplemented by a limited set of assays selected from the investigational phase to allow setting of routine quality parameters. This may involve *in vivo* studies and normally would involve sampling at the times shown below for routine testing.

- **Routine (Local process validation):** Ongoing routine validation using a small set of parameters selected on the basis of the above studies. This will not normally involve *in vivo* studies. Advice may be sought from SACBC on the validation requirements for red cells produced from automated processing of whole blood or other technologies that are not specified in Table 8.2.

Red cell components may be derived either from whole blood or collected by apheresis and, in either case, the standard requirements for donor selection and for mandatory donation microbiological testing should be fulfilled. When well prepared, there is no evidence that the clinical performance of any of these products is different, and the guidance provided below applies equally to the various approaches.

In vitro assays should be performed on samples representative of the pack contents taken by an aseptic technique that does not appreciably alter the gross volume of the pack contents (must be kept to a minimum, but in any case no greater than 10%). Parallel testing of units prepared by a well-established

method is recommended, and the use of a split-pool or crossover design will increase the power of such comparisons. If required, *in vivo* studies, preferably with parallel testing of 'standard' components, should be performed on the last day of the proposed storage period. The number of units studied should be determined by statistical analysis based on the difference between test and control units to be detected. A sample size of at least 12 tests or controls would be required to detect a 30% difference in ATP and potassium at Day 35 of storage using an unpaired study. Fewer units will be required if a pooled and split study design is used, but should not be less than four.

Red cell components will be stored for the recommended storage period or longer in the case of experimental additive solutions (AS) that are designed to extend the shelf life of red blood cells (RBC). Samples will be taken weekly (or minimally at Days 1, 21, 35 and at the end of storage if this is >35 days) for *in vitro* studies. If required, autologous *in vivo* recovery studies should be undertaken at the end of the storage period.

8.2.2: In vitro studies

The measurements described below and in Table 8.2 will be made at the time of component production (Day 0/Day 1) or other relevant stages of component preparation. An equal number of appropriate control components (e.g. standard AS RBC) should be tested in parallel. Greater consistency of information may be obtained if two or more group-compatible components are pooled and divided prior to processing for *in vitro* studies only. The number of units to be studied should be based on the study objectives and design.

8.2.3: On the day of component production/collection

Weight, volume, haematocrit (L/L), haemoglobin (Hb, g/unit), platelets (x 10^9/unit), red cell loss* (%), platelet loss* (%), leucocyte depletion (given as residual WBC × 10^6/unit) and log depletion*. These results should be obtained by validated test procedures and be within the limits defined by the preliminary component specification.

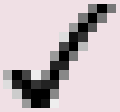
* Relevant to procedures involving integral filtration or other methods that are likely to result in loss of cellular components during production. Validated techniques using flow cytometry or cell counting chambers should be used to count leucodepleted components and would currently be expected to exhibit a sensitivity of less than or equal to 1 leucocyte per microlitre.

Table 8.2 Evaluation of new red cell components for transfusion: recommended tests

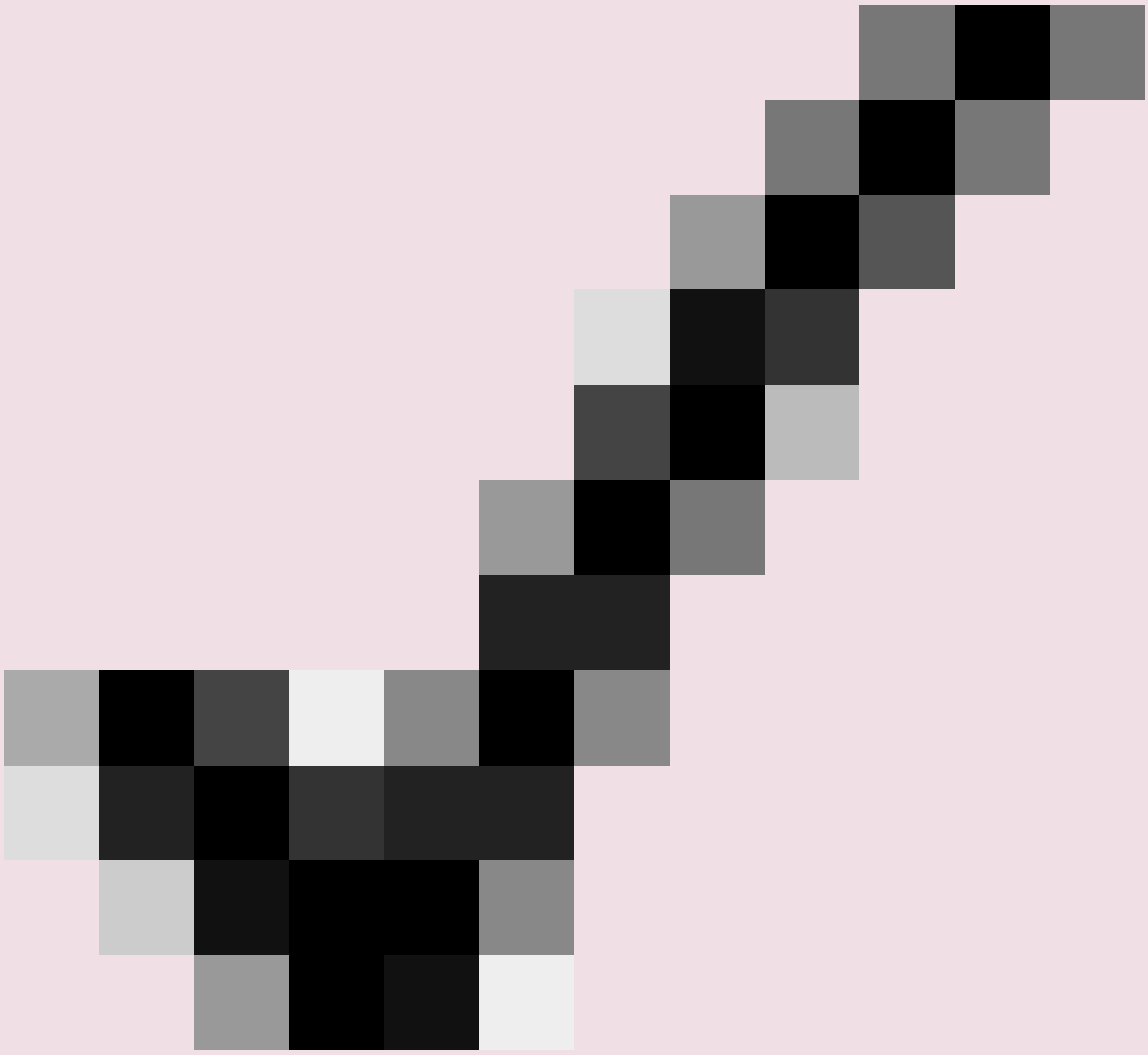
New characteristic parameter	New pack	Leuco-depletion	New centrifugation / component extractor (e.g. Optipress, Compomat etc.)	Novel anti-coagulant	Novel apheresis system	Novel additive solution	Novel plasticiser/ plastic	Irradiation	Pathogen reduction
Unit volume (mL)	✓	✓	✓	✓	✓	✓	✓	✓	✓

Haematocrit (L/L)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Haemoglobin (g/unit)	✓	✓	✓	✓	✓	✓	✓	✓	✓
MCV	✓	✓	✓	✓	✓	✓	✓	✓	✓
WBC (x 10^6 /unit) (post-leucodepletion)		✓			✓		✓		
Leucocyte subsets (%) (post-leucodepletion)		?							
Residual platelets (x 10^9/unit)			✓	✓					
Hb loss (g) (post-filter)		✓	✓						
K^+ (mmol/L)	✓	✓	✓	✓	✓	✓	✓ †	✓	✓
Haemolysis (%)	✓	✓	✓	✓	✓	✓	✓ †	✓	✓

pH				✓		✓	✓	✓	✓
Lactate (mmol /L)				✓		✓	✓	✓	✓
Glucose (mmol /L)				✓		✓	✓	✓	✓
ATP (µmol/g Hb)	✓	✓		✓	✓	✓	✓	✓	✓
2,3-DPG (µmol/g Hb)				✓		✓	✓	✓	✓
Na^+ (mmol/L)				✓		✓	✓	✓	✓
pCO_2 (kPa)				✓		✓	✓	✓	✓
pO_2 (kPa)				✓		✓	✓	✓	✓
Pathogen reduction*	?	✓							
Prion protein (PrPc) and microvesicles		?							?
24-hour recovery (%)				?		✓	✓	?	✓

Recovered plasticiser in supernatant and cells							 †		
Osmotic fragility									
Microvesicles									
Some components may need to be tested for a combination of parameters, e.g. apheresis red cells in a novel /experimental additive solution (AS) that are also leucodepleted. In this case the sampling requirement includes that of a leucodepleted red cell component and that of an experimental AS component. Where novel plasticiser and additive solution are combined, the requirements for novel plasticiser are sufficient to cover both elements.									

Key:



= recommended; ? = optional; other tests are not excluded; *= normally undertaken by the manufacturer; †= also consider the effects of irradiation

At the end of the storage period components should be checked for sterility and a representative sample tested for 24-hour red cell recovery by a validated technique yielding equivalent results to ^{51}Cr labelling methods. Results will be considered acceptable if the mean 24-hour recovery is >75% with a standard deviation of <9%.

8.2.4: During storage

Parameters to be studied during storage of red cells include: haemoglobin, haematocrit, MCV, ATP, 2,3-DPG, glucose, lactate, potassium, haemolysis (soluble haemoglobin as a percentage of total haemoglobin per mL of whole product), pH, pO_2, pCO_2, cytokines. These may include interleukin-1, IL-1, IL-6, IL-8, TNF- and TGF-. Measurements should, wherever possible, be by bioassay (seek advice from SACBC). Cytokine measurements are complex and may be considered optional. As red cell components are leucocyte depleted, measurement of leucocyte-derived cytokines is probably not informative. Advice should be taken from SACBC on the selection of cytokine tests.

These results should be obtained by validated test procedures. Where manipulation of components during processing might increase the risk of bacterial contamination, microbiological sterility testing should be performed at the end of storage.

Consideration should be given when performing *in vitro* studies to including periods where red cells are removed from controlled temperature environment to reflect current practice where red cells may be temporarily stored out with their designated storage temperature. This might include transport of red cells, equipment breakdown or when red cells are issued and collected for transfusion to a patient.

Novel Plasticisers (please also refer to Table 8.2 and section 8.8 for further guidance):
Where novel plasticisers are used, the levels of recovered plasticiser should be monitored over shelf life to assess the levels of leaching into the blood component. Blood bag manufacturers or external laboratories may be required for chemical analysis of plasticisers. Methodology will be specific to the plasticiser under investigation, but likely to be by liquid chromatography-mass spectrometry. Advice can be sought from manufacturers, SACBC and peer-reviewed literature. It is also important to consider metabolites that may also influence product quality and may have toxicological effects. Concentrations in the supernatant and red cells should be measured at the beginning, during and end of storage to assess leaching and potential patient exposure. Consideration must be given to the effects of irradiation on the bag and subsequent leaching potential. Suppliers must undertake toxicology studies as part of CE/UKCA/UKNI marking. Suppliers must provide evidence of an independent review of toxicology data; this data will then be reviewed by SACBC.

Novel Additive Solutions (please also refer to section 8.8 for further guidance):
Where novel additive solutions are used, effects on storage must also be taken into account. Table 8.2 provides a list of recommended tests. Where there are combinations of novel elements to a blood bag system (e.g. novel plasticiser and additive solution), then Table 8.2 should be used to ensure requirements for each element is included within the minimum recommended tests. There will likely be overlap in requirements and SACBC can provide advice on this if required.

8.2.5: Autologous in vivo studies

See Table 8.2 for details of testing. An equal number of appropriate control components obtained from healthy volunteer donors with ethical approval (e.g. standard AS RBC) should be tested in parallel. The number of components transfused should be justified based on the study objectives and design.

8.3: Evaluation of new platelet components for transfusion

8.3.1: Introduction

In establishing any novel component, the development process is expected to involve three stages (see Table 8.1d):

- **Investigation (Phase 0):** Initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10 -16) to establish concepts. This should involve *in vitro* studies with serial sampling, and may also involve *in vivo* studies. Components produced during this phase should not be used for transfusion. For clarity the guidance on which tests need to be performed is as shown in Tables 8.3a and 8.3b.

- **Validation (Phase 1/Phase 2):** Operational validation on a larger number of units (e.g. 125) to establish routine operation of the technique, normally testing for those parameters listed in the current edition of the Red Book. These tests may be supplemented by a limited set of assays

selected from the investigational phase to allow setting of routine quality parameters. This may involve *in vivo* studies and normally would involve sampling at the times shown below for routine testing.

- **Routine (Local process validation):** Ongoing routine validation using a small set of parameters selected on the basis of the above studies. This will not normally involve *in vivo* studies. Advice may be sought from SACBC on the validation requirements for platelets produced from automated processing of whole blood or buffy coats or other technologies that are not specified in Tables 8.3a and 8.3b.

Platelet components may be derived from whole blood using platelet-rich plasma or buffy coat methods of preparation, or by plateletpheresis and, in either case, the standard requirements for donor selection and for mandatory donation microbiological testing should be fulfilled. For components prepared in a closed system, storage in specifically designed plastic bags is currently undertaken with gentle agitation for up to 7 days at 22 ±2°C. The platelet concentration should not exceed the platelet bag manufacturer's recommendation. Platelet components may be subjected to leucodepletion, storage in platelet additive solutions in place of plasma and, in the case of whole blood derived components, pooling of four to six units to form an adult equivalent dose. When well prepared, there is no evidence that the clinical performance of any of these products is different, and the guidance provided below applies equally to the various approaches.

In vitro assays should be performed on samples representative of the pack contents taken by an aseptic technique that does not appreciably alter the gross volume of the pack contents (must be kept to a minimum but in any case no greater than 10%) on Days 1, 3, 5 and 7 (and further samples if an extension of shelf life is proposed or for components that have a shorter shelf life). For studies investigating an extension to shelf life, consideration should be given to testing the component 1 day after the proposed limit of shelf life (e.g. Day 8 for a 7-day shelf life). Parallel testing of units prepared by a well-established method is recommended, and the use of a split-pool or crossover design will increase the power of such comparisons. *In vivo* studies, preferably with parallel testing of 'standard' components, should be performed on the last day of the proposed storage period. The number of units to be studied should be based on the study objectives and design and determined by statistical analysis based on the difference between test and control units to be detected. A sample size of ten tests or controls would be required to detect a 30% difference in pH and CD62P at Day 7 of storage using an unpaired study. Fewer units will be required if a pooled and split study design is used.

8.3.2: Investigational phase

8.3.2.1: Guidance

Table 8.3a recommends an assessment format for different kinds of novel development that may be expected for platelet components. While these are listed against the recommended assays above, this is not intended to be restrictive and comparable alternatives may be employed. It is recommended that any protocol for the evaluation of a novel blood component or production method be discussed with the Chair of the SACBC before finalisation.

For leucodepleted components, leucocyte enumeration should involve validated techniques and would currently be expected to exhibit a sensitivity of less than or equal to 1 leucocyte per microlitre.

8.3.3: In vitro assessment

8.3.3.1: Background

In vitro assessments essentially use surrogate assays that are hoped to be indicative of the *in vivo* performance of platelets, as measured by haemostatic effect, *in vivo* recovery and survival and corrected count increment following transfusion. While a large number of *in vitro* assays have been proposed, only a few of these have been shown to correlate with post-transfusion indices. This area has been reviewed by the BEST group[1] and can be summarised in Table 8.3a (* = correlates with *in vivo* viability).

Any platelet production system that may be considered as having the potential for an increased risk of bacterial contamination or growth should include an assessment of sterility as part of the initial validation phase. It is recommended that at least 50 apheresis units or pools (each sufficient for a standard adult dose) should be assessed for sterility by a validated technique prior to *in vivo* assessment and routine introduction of the component into clinical use.

Consideration should be given when performing *in vitro* studies to including periods where platelets are temporarily not agitated to reflect current practice such as during transportation.

Novel Plasticisers (please also refer to Tables 8.3a, 8.3b and section 8.8 for further guidance):
Where novel plasticisers are used, the levels of recovered plasticiser should be monitored over shelf life to assess the levels of leaching into the blood component. Blood bag manufacturers or external laboratories may be required for chemical analysis of plasticisers. Methodology will be specific to the plasticiser under investigation, but likely to be by liquid chromatography-mass spectrometry. Advice can be sought from manufacturers, SACBC and peer-reviewed literature. It is also important to consider metabolites that may also influence product quality and may have toxicological effects. Concentrations in the supernatant and platelets should be measured at the beginning, during and end of storage to assess leaching and potential patient exposure. Consideration must be given to the effects of irradiation on the bag and subsequent leaching potential. Suppliers must undertake toxicology studies as part of CE/UKCA/UKNI marking. Suppliers must provide evidence of an independent review of toxicology data; this data will then be reviewed by SACBC.

Novel Additive solutions (please also refer to section 8.8 for further guidance):
Where novel additive solutions are used, effects on storage must also be taken into account. Table 8.3b provides a list of recommended tests. Where there are combinations of novel elements to a blood bag system (e.g. novel plasticiser and additive solution), then Table 8.3a and 8.3b should be used to ensure requirements for each element is included within the minimum recommended tests. There will likely be overlap in requirements and SACBC can provide advice on this if required.

Table 8.3a In vitro assessment

	Recommended	Alternatives (may be used if validated against parameters that correlate with in vivo viability)
(a)	Product content Volume Platelet content Leucocyte content Plasma content (for additive developments only)	
(b)	Platelet morphology (proportion of discs)	

	Determination of swirling Morphology index (phase microscopy)* Extent of shape change by ADP*	
(c)	Platelet metabolism ATP* Hypotonic shock response* pO_2/pCO_2 pH Glucose consumption Lactate production	
(d)	Extent of platelet activation P-selectin (CD62P) on platelet surface and in supernatant Beta thromboglobulin release	Surface GPIb/IX (CD42a/42b) Surface GPIIb/IIIa (CD41/CD61) Platelet fibrinogen binding Serotonin content or release Glycocalicin or PF4 release Annexin V binding (to phospholipid)
(e)	Extent of platelet lysis Supernatant lactate dehydrogenase	Soluble annexin V
(f)	Measurements reflecting in vitro function Aggregation in response to paired antagonists (e.g. 80 µM ADP and 8 µg/mL collagen)	In vitro bleeding time (in development) Platelet adhesion (e.g. Baumgartner)
(g)	Assays indicative of possible side effects Cytokines/chemokines, particularly platelet-derived (IL-6, IL-8, RANTES, TNF-, TGF-): optional, (if performed bioassay is preferable to immunoassay) FXIIa formation (particularly for novel plastics or filters)	

	Bacterial contamination (at end of shelf life only) Pathogen reduction (for these processes only)	

8.3.4: In vivo assessment

If *in vivo* assessment is required local ethical committee approval should be obtained prior to commencing the *in vivo* assessment.

Additional measurements at 4–6 and/or 24 hours post-transfusion may give some indication of platelet survival.

Any *in vivo* assessments should be performed at the end of the proposed storage period, following generation of sufficiently reassuring data from *in vitro* studies. For studies investigating an extension to shelf life, consideration should be given to testing the component 1 day after the proposed limit of shelf life (e.g. Day 8 for a 7-day shelf life). Due to the inherent variability of patients, use of a crossover design or dual labelling technique in stable, afebrile thrombocytopenic patients without evidence of platelet consumption (or in volunteers) is strongly recommended so that each patient acts as their own control. The number of components transfused should be justified on the basis of the study objectives and design.

Platelet counts should be assessed immediately prior to infusion of an appropriate dose of ABO identical platelets and 1 hour post-infusion.

Two approaches are established:

- Use of radioisotope-labelled platelets in normal volunteers for determination of platelet recovery and survival: This approach is not applicable to pooled products. Validated techniques must be used.
- Determination of recovery after transfusion: An appropriate adult dose (>240 × 10^9 platelets) of ABO identical platelets may be used to determine increments and therapeutic effect (bleeding time measurements are not recommended). Patients known or suspected to have lymphocytotoxic or human platelet antigen (HPA) antibodies should be excluded and should have no evidence of hypersplenism, sepsis, ongoing haemorrhage or other cause of increased platelet consumption.

Table 8.3b Evaluation of new platelet components for transfusion

Parameter	Leuco-depletion	Pathogen reduction	Extended storage	Sterile connection	New bag, additive or anticoagulant	Novel plasticiser /plastic
Volume (d1)	✓	✓	✓	✓	✓	✓
Platelet content	✓	✓	✓	✓	✓	✓
Red cell count	✓	✓	✓		✓	✓
Leucocyte content (d1)	✓	?	?		?	✓
Leucocyte subsets (%)	?	?	?		?	

Plasma : PAS ratio					✓	
Plasma content					✓	
Morphology, e.g. Swirl test	✓	✓	✓	✓	✓	✓
Activation, e.g. beta thromboglobulin, CD62P	✓	✓	✓		✓	✓
Lysis, e.g. lactate dehydrogenase	✓	✓	✓		✓	✓
Metabolic activity, e.g. ATP, pH, Lactate, Glucose, pCO_2, pO_2	✓	✓	✓		✓	✓
Function e.g. Aggregation	?	?	?	?	?	✓
Cytokines/chemokines	✓	✓	✓		✓	✓
FXIIa	?	?			?	✓
Sterility	if dock on	✓	✓	✓	?	✓
PrPc and microvesicles	?					✓
Pathogen reduction*	?	✓				
Recovered plasticiser in supernatant and plasma						✓ †

Key: ✓= recommended; ? = optional; other tests are not excluded; * = normally undertaken by the manufacturer; †= also consider the effects of irradiation.

Planned studies may fall into more than one category in which case all indicated assays should be performed. d1 = Day 1.

Where novel plasticiser and additive solution are combined, the requirements for novel plasticiser are sufficient to cover both elements.

8.4: Evaluation of new fresh frozen plasma/cryoprecipitate components for transfusion

8.4.1: Introduction

In establishing any novel component, the development process is expected to involve three stages (see Table 8.1d):

- **Investigation (Phase 0):** Initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10 -16) to establish concepts. This should involve *in vitro* studies with serial sampling, and may also involve *in vivo* studies. Components produced during this phase should not be used for transfusion. For clarity the guidance on which tests need to be performed is as shown in Table 8.4.

- **Validation (Phase 1/Phase 2):** Operational validation on a larger number of units (e.g. 125) to establish routine operation of the technique, normally testing for those parameters listed in the current edition of the Red Book. These tests may be supplemented by a limited set of assays selected from the investigational phase to allow setting of routine quality parameters. This may involve *in vivo* studies and normally would involve sampling at the times shown below for routine testing.

- **Routine (Local process validation):** Ongoing routine validation using a small set of parameters selected on the basis of the above studies. This will not normally involve *in vivo* studies. Advice may be sought from SACBC on the validation requirements for plasma components produced from automated processing of whole blood or other technologies that are not specified in Table 8.4.

8.4.2: In vitro evaluation of novel fresh frozen plasma

8.4.2.1: Suggested study design

Because of the wide normal range of some clotting factors and potential inter-batch variation of assays, it is suggested that novel units and controls be produced and assayed in parallel, with the novel technology being the only variable. A less costly alternative, if logistics permit, is to do a pooled paired comparison, where two units are pooled, and one half processed by the novel technique. This provides greater statistical power for fewer units assayed, and is particularly important for storage studies. Since levels of FVIII and von Willebrand factor are ABO group-dependent, investigators should consider an equal mix of group A and O donations in the experimental design. The number of units to be studied should be based on the study objectives and design, and determined by statistical analysis based on the difference between test and control units to be detected. A sample size of at least 16 test or controls would be required to detect a 30% difference in FVIII levels using an unpaired study. Fewer units will be required if a pooled and split study design is used.

For leucocyte depleted or pathogen reduction systems it is recommended that assays are performed on samples collected before and after the process under investigation. Ideally provision should be made for storing and testing aliquots from each pack at every time point, as thawing out three or four different packs at each time point introduces excessive variation. However, a pre-validation should be done to ensure that the behaviour of the aliquoted component during storage is the same as that in the main pack.

8.4.2.2: Assays required

The extent of any evaluation depends in part on the degree of novelty of the component. The list of assays below need not be applied in every setting. Table 8.4 gives a summary of which assays are recommended

in different situations. Advice may be sought from SACBC on the validation requirements for plasma components produced from automated processing of whole blood or other technology that is not specified in Table 8.4.

All evaluations must include the routine quality control parameters such as FVIII:C.
Before freezing:

- volume, platelet count, WBC*, RBC, total protein
- prothrombin time (PT), activated partial thromboplastin time (APTT)
- factors I (fibrinogen), II, V, VII, VIII, IX, X, XI, XIII, von Willebrand factor (vWf):Ag, vWf:RiCof, which measures the functional activity or an assay validated as yielding equivalent results, vWf multimeric analysis, vWF cleaving protease
- inhibitors of coagulation – antithrombin, protein C, protein S, 2-antiplasmin
- markers of unwanted activation of coagulation* – prothrombin fragment 1.2, fibrinopeptide A, factor XIIa, thrombin-antithrombin (TAT) complexes.

*Particularly relevant to plasma which has been collected by any filtration technique, in which case the assays should be performed before and after filtration or to packs made of novel materials.

During storage:

- Consideration should be given to performing storage studies at >=20°C in addition to those at <=30° C to reflect hospital storage conditions. Samples should be taken at 12 and 24 months. Ideally, all clotting factors should be assayed at each time point, if only in a few packs. FVIII should be assayed at each time point as a minimum in addition to the proteins most severely affected by the initial process.
- Storage parameters may be assayed after the date of implementation of routine production, provided data ‘keep ahead’ of the age of any clinical product which might be issued.

Novel Plasticisers (please also refer to Table 8.4 and section 8.8 for further guidance):
Where novel plasticisers are used, the levels of recovered plasticiser should be monitored over shelf life to assess the levels of leaching into the blood component. Blood bag manufacturers or external laboratories may be required for chemical analysis of plasticisers. Methodology will be specific to the plasticiser under investigation, but likely to be by liquid chromatography-mass spectrometry. Advice can be sought from manufacturers, SACBC and peer-reviewed literature. It is also important to consider metabolites that may also influence product quality and may have toxicological effects. Concentrations in the plasma should be measured at the beginning, during and end of storage to assess leaching and potential patient exposure. Consideration must be given to the effects of irradiation on the bag and subsequent leaching potential. Suppliers must undertake toxicology studies as part of CE/UKCA/UKNI marking. Suppliers must provide evidence of an independent review of toxicology data; this data will then be reviewed by SACBC.

8.4.3: In vitro evaluation of novel cryoprecipitate

It is assumed that this will be produced from a ‘novel’ start plasma so that investigators will be aware of any specific losses of clotting factors which should be particularly considered.
Assays to be performed before and after production, and during storage: fibrinogen, FVIII:C.

8.4.4: Cryosupernatant

The only clinical indication for this component is for plasma exchange procedures for patients with thrombotic thrombocytopenic purpura. Analysis of vWf multimers and cleaving protease is therefore appropriate. vWf multimeric and cleaving protease analysis should be performed in a laboratory recognised to be proficient in this technique and which is performing the assay regularly.

Table 8.4 Evaluation of novel plasma components

	Fresh frozen plasma							Cryo-precipitate	C supe
Parameter	Novel filter	New centrifuge/ component extractor	Novel anticoagulant	Novel plasticiser /plastic	Novel apheresis system	Novel apheresis + anticoagulant	Pathogen reduction		
Volume	✓	✓	✓	✓	✓	✓	✓	✓	✓
Leucocyte content	✓	✓	✓	✓	✓	✓			–
FVIII:C	✓	✓	✓	✓	✓	✓	✓	✓	–
Platelets	✓	✓	✓	✓	✓	✓	✓	–	–
PT ratio	✓	–	✓	✓	–	✓	✓	–	–
ROTEM /ROTEG	✓	–	✓	✓	–	✓	✓	–	–
APTT ratio	✓	–	✓	✓	–	✓	✓	–	–
Fibrinogen	✓	–	✓	✓	–	✓	✓	✓	–
II, V, VII, IX, X, XI, XIII	✓	–	✓	✓	–	✓	✓	–	–
vWf:Ag	✓	–	✓	✓	–	✓	✓		–
vWf:RiCof	✓	–	✓	✓	–	✓	✓		
AT III, Prot C, Prot S	✓	–	✓	✓	–	✓	✓	–	–
TAT/Frag1.2 /FPA + FXIIa	✓	–	✓	✓	✓	✓	✓	Omit if not eleva source plasma	
C1 inhibitor	✓	–	✓	✓	–	✓	✓		–
vWf multimers	✓	–	✓	✓	–	✓	✓		✓

vWF cleaving protease	✓	–	✓	✓	–	✓	✓		✓
Alpha-2 anti-plasmin	✓	–	✓	✓	–	✓	✓		–
Pathogen reduction*							✓		–
PrPc /microvesicles	?	–							–
Clinical trial	–	–	#		#	#	✓*	#	#
Recovered plasticiser in supernatant				✓					
Key: ✓ = recommended; – = not needed; # = consider individually. * = normally undertaken by the manufacturer.									

8.4.5: In vivo studies

Whether or not *in vivo* studies are needed depends on the degree of novelty of the component, e.g. this may not be necessary for plasma which has been leucocyte depleted in the course of producing leucocyte-depleted red cells, but would certainly apply in the case of a novel pathogen reduced plasma which had been exposed to chemicals. Unlike red cells and platelets, administration to normal volunteers has not been traditional. Suitable patient groups to consider would be:

For fresh frozen plasma:

- correction of prolonged international normalised ratio (INR) prior to liver biopsy
- liver transplant recipients
- plasma exchange for thrombotic thrombocytopenic purpura (TTP)
- disseminated intravascular coagulation (DIC).

It is difficult to get permission to study neonates and usually considerable experience has to have been gained with the product in adults.

A randomised design is preferred, with standard fresh frozen plasma as control.

For cryoprecipitate:

- DIC
- liver disease/transplant
- congenital hypofibrinogenaemia, if maintained on cryoprecipitate.

8.5: Evaluation of plasma for fractionation for the manufacture of immunoglobulin

8.5.1: Introduction

In establishing any novel component, the development process is expected to involve three stages (see Table 8.1d):

- I**nvestigation (Phase 0):** Initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10 -16) to establish concepts. This should involve *in vitro* studies with serial sampling. Components produced during this phase should not be used for fractionation. For clarity, the guidance on which tests need to be performed is shown in Table 8.5.
- **Validation (Phase 1/Phase 2):** Operational validation on a larger number of units (e.g. 125) to establish routine operation of the technique, using a small set of parameters selected on the basis of the above studies to allow setting of routine quality parameters. It may not be necessary to test all subset parameters on the full number of units and a risk-based approach should be used if the number of tests for a given parameter is going to be reduced.
- **Routine (Local process validation):** Ongoing routine validation using parameters selected on the basis of the above studies. Advice may be sought from SACBC on the validation requirements for plasma for the manufacture of immunoglobulin produced from automated processing of whole blood or other technologies that are not specified in Table 8.5.

  The blood services' fractionation partner may have additional requirements.

8.5.2: In vitro evaluation of novel frozen plasma for fractionation

8.5.2.1: Suggested study design

Because of the wide normal range of some plasma proteins and potential inter-batch variation of assays, it is suggested that novel units and controls be produced and assayed in parallel, with the novel technology being the only variable. A less costly alternative, if logistics permit, is to do a pooled paired comparison, where two units are pooled, and one half processed by the novel technique. This provides greater statistical power for fewer units assayed, and is particularly important for storage studies. As it may not be appropriate to compare plasmapheresis and whole blood derived plasma, data could be compared against manufacturer's claims or published literature where there isn't a comparable plasmapheresis process in place.

The number of units to be studied should be based on the study objectives and design and determined by statistical analysis based on the difference between test and control units to be detected. A sample size of at least 16 test or controls is suggested for the evaluation of new fresh frozen plasma/cryoprecipitate components for transfusion and this same sample size is suggested here if using an unpaired study. Fewer units (>=10) will be required if a pooled and split study design is used, however this approach will not be suitable for all studies, e.g. for studies looking at contact activation where individual donor susceptibility is

important. While ABO group may not be as relevant here as when levels of FVIII and von Willebrand factor are considerations, it is recommended that validation should include an equal mix of group O and non-group O donations.

Ideally provision should be made for storing and testing paired units or aliquots from each unit at every time point, as thawing out three or four different packs at each time point introduces excessive variation. However, a pre-validation should be done to ensure that the behaviour of the aliquoted component during storage is the same as that in full sized units.

8.5.2.2: Assays required

The extent of any evaluation depends in part on the degree of novelty of the method used to collect plasma. Table 8.5 gives a summary of which assays are recommended in different situations. Advice may be sought from SACBC on the validation requirements for plasma components for fractionation produced from automated processing of whole blood or for the manufacture of other blood products not specified in Table 8.5. Sections "8.4 Evaluation of new fresh frozen plasma/cryoprecipitate components for transfusion" and "8.6 Generic protocol for the evaluation of apheresis equipment" should also be consulted if the collection device is new and/or intended for multiple purposes e.g. plasma for fractionation and clinical plasma (FFP). Where appropriate, methods must be those recommended by the European pharmacopeia or a validated equivalent method, for example total protein.

Before freezing:

- volume, platelet count, WBC*, RBC

*Particularly relevant to plasma which has been collected by any filtration technique. If filtration is done outside the collection procedure, assays should be performed before and after filtration.

During storage:

- Samples should ideally be tested after one to four weeks of frozen storage at <=-20°C freezing to assess the quality of frozen plasma. Tests should be those presented in Table 8.5 and include:
    - total protein, Albumin, IgG
    - markers of unwanted activation of coagulation e.g. prothrombin fragment 1.2, fibrinopeptide A, factor XIIa, thrombin-antithrombin (TAT) complexes. It is important to measure markers of activation for the manufacture of immunoglobulins as there have been reports of batches of immunoglobulins ‘contaminated’ with FXIa, resulting in thrombotic events
    - optional tests – prothrombin time (PT), activated partial thromboplastin time (APTT) and FVII, FVIII and Fibrinogen. These tests are not relevant for the manufacture of immunoglobulins but should be considered to future proof against the use of plasma for the manufacture of labile proteins such as clotting factors. A complete validation would be required before fractionation to produce clotting factor concentrates could commence.

Table 8.5 Evaluation of novel plasma for the manufacture of immunoglobulin

Table 8.5 gives a summary of which assays are recommended for the manufacture of immunoglobulins only. Advice may be sought from SACBC on the validation requirements for plasma for the manufacture of labile proteins such as clotting factors.

Assessment [1,2]	Whole blood derived	Apheresis derived	Novel filter
Volume[3]	✓	✓	✓
Leucocyte content[3]	✓	✓	✓
Platelets[3]	✓	✓	✓
Red cells[3]	✓	✓	✓
Plasma Haemoglobin[4]	?	?	?
Total protein	✓	✓	✓
Albumin	✓	✓	✓
IgG	✓	✓	✓
Markers of unwanted activation of coagulation[5]e.g. TAT, Frag1.2, FPA, FXIIa, FVIIa, FXIa, C1 inhibitor, S2302	✓	✓	✓
PT ratio, APTT ratio[6]	?	?	?
FVII, FVIII, Fibrinogen[6]	?	?	?
Complement C3a, C5a[4]	?	?	?

[1]Where appropriate, methods must be those recommended by the European pharmacopeia or a validated equivalent method
[2]Additional assays may need to be performed to comply with requirements from fractionators
[3]Tests are only required prior to freezing
[4]Parameter may be required by fractionators – needs to be confirmed. If required parameter will become mandatory
[5]Included due to reports of batches of immunoglobulins ‘contaminated’ with FXIa, resulting in thrombotic events
[6]Recommended to future proof against the use of plasma for the manufacture of labile proteins such as clotting factors

8.6: Generic protocol for the evaluation of apheresis equipment

This protocol sets out the minimum requirements for new apheresis equipment and, in a generic form, the mechanism for assessing acceptability of the equipment hardware, the software and the associated apheresis sets. The specific validation or trial of apheresis collections from new equipment is covered in section 8.7. Novel components, as defined in section 8.1, produced as a result of new equipment will be assessed as detailed in other sections of this chapter.

8.6.1: Minimum requirements

8.6.1.1: General

Equipment should be CE/UKCA/UKNI marked or the Blood Service should participate to facilitate CE/UKCA /UKNI marking.
Manufacturers must comply with Good Automated Manufacturing Practice (GAMP).[2]

8.6.1.2: Equipment hardware

Equipment should contain the following:

- manual override system
- blood flow monitor
- in-line air detector
- integral blood filter
- anticoagulant flow indicator
- collection volume preset device
- visual audible alarm for procedure completion
- automatic standby mode for power failure
- power up self-check to include all critical safety and operational procedures.

8.6.1.3: Equipment hardware

Software should provide parameters:

- for accepted total blood volume calculation algorithm
- for accepted citrate reinfusion rate calculation algorithm
- for fixed upper limit citrate reinfusion (see Chapter 5)
- for programmable upper limit total collection volume
- must not exceed predetermined fluid reinfusion limits (e.g. citrate, saline)
- for alarm and prevent use of incorrect set (incongruent) for programmed procedure
- prevent procedure where predicted post-collection parameters fall outside programmable safety limits as defined in Chapter 5.

For other measures, see Chapter 5.

8.6.1.4: Apheresis sets

Apheresis sets should have:

- a closed system
- a visual system to minimise risk of transposition of fluid lines
- a microbial filter on ‘spiked’ lines (unless the set will only be used to collect plasma for fractionation)
- a diversion line and pouch for sampling
- a means of preventing incorrect connections to the set for IV fluid (e.g. saline) and anticoagulant.

Consideration should be given to the incorporation of a pouch on the final pack to facilitate bacterial contamination testing.

For other measures, see Chapter 5.

The overall mechanism for equipment acceptance is given in Figure 8.1 for reference. Validation, installation qualification, operational qualification and performance qualification would be defined by the Blood Service, taking account of the advice within these guidelines.

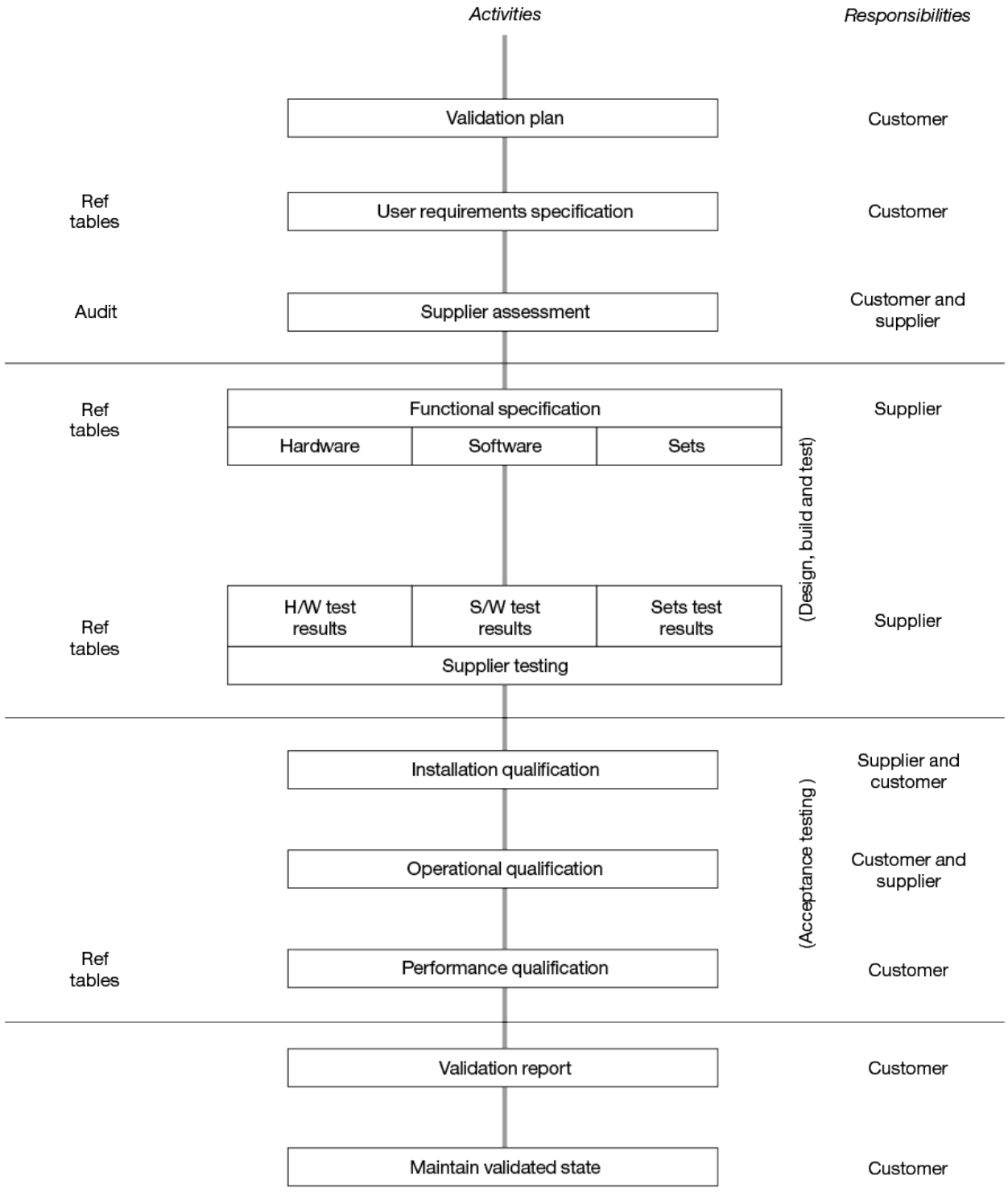


Figure 8.1 Generic flowchart of apheresis equipment acceptance

8.7: Generic protocol for the evaluation of blood packs for whole blood donations and apheresis collections

8.7.1: Introduction

This protocol sets out in generic form the essential features of blood pack evaluations as required by the UK Blood Transfusion Services. National Services should exercise discretion in the extent to which the protocol should be applied; assessment of degree of novelty will assist in this (see table 8.1b). It may be appropriate to consider an abbreviated format, e.g. when the change to be evaluated represents the attachment of a filter to a pack assembly that is already in routine use, or where the change consists of a modified port access design. Section 8.8 provides additional guidance for the evaluation of novel plasticisers and additive solutions, where they are combined.

The protocol is not intended for use with packs for stem cell collection and storage, although the principles outlined may be helpful. The principles of this section apply to components produced from whole blood donations as well as whole blood itself and components from apheresis collections.

8.7.2: General principles

Each trial will be fully documented and will have a unique trial reference number. The key requirements are as follows:

- An evaluation outline: What type of pack is being investigated, where, when and the standards against which the assessment will be based.

- The evaluation objective: To demonstrate the packs are and remain free from defects and are suitable for the production and storage of components that meet current guidelines.

- Identification of any restrictions, e.g.:
    - situations where an evaluation would be required
    - agreement on ownership and release of the evaluation report with the supplier/manufacturer
    - limitations of the report and its distribution.

- How the trial will be controlled, e.g.:
    - the identity of the person/persons responsible for the trial and their reporting lines
    - sign-off procedures and authorities including concessionary changes
    - the trial protocol will be agreed with the supplier and any concessionary changes will require agreement in accordance with local procedures
    - trials will be conducted in three phases. Satisfactory performance and sign-off in Phase 0 is a prerequisite to progression to Phase 1 and satisfactory performance in Phase 1 is a prerequisite to progression to Phase 2
    - blood collected in Phase 0 will not be used for transfusion
    - all components prepared in Phase 1 will be subject to routine quality monitoring tests
    - components prepared in Phase 2 will be subjected to routine quality monitoring tests as defined in Table 8.1d and Chapter 7
    - any testing that exceeds the minimum set out herein must be fully incorporated within the report.

- Confidentiality: Any data collected will normally be the property of the organisation performing the trial; blood pack suppliers/manufacturers who wish to release information arising from the trial will require confirmation in writing from the organisation performing the trial that they may do so.

- Quality monitoring: It is expected that packs evaluated under the trial protocol will be subject to routine quality monitoring and reporting procedures, e.g. pack faults, compliance with component specifications etc. It follows that any adverse findings during the trial would generate a corrective action.

A summary of the numbers to be tested for each evaluation or validation phase is given in Table 8.1d. The numbers given are the minimum required. More detail is given in the relevant sections below. Deviations from this number must be agreed in accordance with local procedures and consistent with section 8.7.1.

8.7.3: Phase 0: Evaluation

After an initial familiarisation with novel bag/filters (pre-Phase 0) the purpose of Phase 0 studies is to:

- assess suitability to progress to Phase 1

- determine suitable quality monitoring parameters

- disclose any quality problems that might prevent components collected or prepared in these packs from being used for transfusion.

Processing conditions used in the Phase 0 evaluation should be the same as those applied to Phase 1 and 2 evaluations.

8.7.3.1: Component quality monitoring

Starting donations and all final components will be tested for compliance with relevant parameters listed in the component specifications in these guidelines. Where relevant, additional assays should be performed as specified in the Red Book generic evaluation protocols for new or novel blood components and consistent with sections 8.2 - 8.6.

- Check for minimum / maximum volume or concentration limits (e.g. platelet count) stipulated for the blood bag system. These may vary between systems and should be built into validation tests.

8.7.3.2: Goods inward inspection

- Check that appropriate storage information is shown on the packaging.

- Check the condition of packaging on receipt. Document damaged cartons and examine contents to assess the extent of any damage.

8.7.3.3: Quality assurance pack conformance inspection

Unless otherwise indicated, the following inspection will be performed and documented for all packs to be used in Phase 0 of the trial:

- pack batch number (eye-readable and machine-readable)

- pack type number (eye-readable and machine-readable)

- base label integrity and compliance with Chapter 26 of these guidelines for the uniform labelling of blood and blood components

- base label adherence (a sample of 20 at each temperature)
    - +22°C for 1 week
    - +4°C for 1 week
    - +4°C for 1 day, followed by –25°C for 1 week

- donation number, component type and blood group label adherence (a sample of 20 at each temperature)
    - +22°C for 1 week
    - +4°C for 1 week
    - +4°C for 1 day, followed by –25°C for 1 week

- seals, seams and welds satisfactory
    - absence of leaks
    - anticoagulant/additive free from turbidity, particulate matter and inclusions

- if the inspection requires removal of packs from their overwraps, either repackage and use according to the manufacturer's instructions or perform the examination immediately prior to donation

- check for acceptable handling and storage characteristics of unopened cartons of packs from receipt, through storage to use at sessions.

8.7.3.4: Checks to be performed by collection teams

Collection teams will follow routine procedures for recording pack faults, but additionally should comment on:

- ease of overwrap opening

- integrity of overwrap

- accuracy of instructions for use at time of collection

- acceptability of needle characteristics

- suitability of tubing (length and flexibility)

- general suitability.

8.7.3.5: Checks to be performed by processing team

The processing team will follow routine procedures for recording pack faults, but may additionally wish to comment on:

- breakage rates following freezing

- heat seal failures (in-house seals)

- suitability of tubing (length and flexibility)

- ease of cannula breakage

- ability to sterile dock (during secondary processing)
- integrity of join, following local, current, procedure
- compatibility with instructions for device for sterile connection
- assess compatibility with current protocol for packaging of frozen packs
- inspection of packs after overnight storage at 4°C.

When the minimum number of packs has been evaluated, the individual or group responsible for the trial will prepare and submit a Phase 0 report.

8.7.4: Phase 1: Validation

The purpose of this phase is to allow:

- staff to familiarise themselves with the packs and any associated equipment
- the generation of quality monitoring data
- the development of an appreciation of the suitability of the packs for routine use, i.e. progression to Phase 2 trial.

Phase 1 of the validation process normally will require not less than 125 packs to be tested at the centre undertaking the trial. Deviations from this number must be agreed in accordance with local procedures.

It is expected that a smaller number of packs will be used for familiarisation in other centres. This phase will include the finalisation of standard operating procedures (SOPs) for use in Phase 2.

Blood components produced during Phase 1 may be used therapeutically where they comply with appropriate release criteria.

8.7.4.1: Component quality monitoring

Starting donations and all final components will be tested for compliance with relevant parameters listed in the component specifications in these guidelines.

8.7.4.2: Goods inward inspection

- Check that appropriate storage information is shown on the packaging.
- Check the condition of packaging on receipt. Document damaged cartons and examine contents to assess the extent of any damage.

8.7.4.3: Quality assurance pack conformance inspection

Unless otherwise indicated, the following inspection will be performed and documented for all packs to be used in Phase 1 of the trial:

- pack batch number (eye-readable and machine-readable)
- pack type number (eye-readable and machine-readable)

- base label integrity
- seals, seams and welds satisfactory
    - absence of leaks
    - anticoagulant/additive free from turbidity, particulate matter and inclusions
- if the inspection requires removal of packs from their overwraps, either repackage and use according to the manufacturer's instructions or discard
- check for acceptable handling and storage characteristics of unopened cartons of packs from receipt, through storage to use at sessions.

8.7.4.4: Checks to be performed by collection teams

Collection teams will follow routine procedures for recording pack faults, but additionally should comment on:

- ease of overwrap opening
- integrity of overwrap
- accuracy of instructions for use at time of collection
- acceptability of needle characteristics
- suitability of tubing (length and flexibility)
- general suitability.

8.7.4.5: Checks to be performed by processing team

The processing team will follow routine procedures for recording pack faults, but may additionally wish to comment on:

- breakage rates following freezing
- heat seal failures (in-house seals)
- suitability of tubing (length and flexibility)
- ease of cannula breakage
- ability to sterile dock (during secondary processing)
- integrity of join, following local, current, procedure
- compatibility with instructions for device for sterile connection
- assess compatibility with current protocol for packaging of frozen packs
- inspection of packs after overnight storage at 4°C.

8.7.4.6: End users

Set up a process by which users will feedback information on acceptability of the packs for use. This would involve blood bank and ward/theatre staff. Obtain details on:
Blood bank issues:

- acceptability to end users
- acceptability of number and condition of bleed line samples
- crossmatch/other label adherence
- leak and breakage rates.

Ward/theatre staff issues:

- general acceptability
- accessibility of ports for giving sets
- leak and breakage rates.

When the minimum number of packs has been evaluated, the individual or group responsible for the trial will prepare and submit a Phase 1 report.

8.7.5: Phase 2: Evaluation

A minimum of 2000 packs from each of two batches for whole blood collection processes or 300 sets for apheresis collection will be used in this phase to allow data on consistency of manufacture to be collected.

Relevant SOPs will be available before commencing Phase 2. Customer communication and any associated training will also have been done by this date.

Blood components produced during Phase 2 may be used therapeutically where they comply with the normal release criteria.

8.7.5.1: Goods inward inspection

- Check that appropriate storage information is shown on the packaging.
- Check the condition of packaging on receipt. Document damaged cartons and examine contents to assess the extent of any damage.

8.7.5.2: Quality assurance pack conformance inspection

Unless otherwise indicated, the following inspection will be performed and documented for packs to be used in Phase 2 of the trial:

- pack batch number (eye-readable and machine-readable)
- pack type number (eye-readable and machine-readable)
- base label integrity
- seals, seams and welds satisfactory

- absence of leaks
- anticoagulant/additive free from turbidity, particulate matter and inclusions.

8.7.5.3: Checks to be performed by collection teams

Collection teams will follow routine procedures for recording pack faults, but additionally should comment on:

- ease of overwrap opening
- integrity of overwrap
- accuracy of instructions for use at time of collection
- acceptability of needle characteristics
- suitability of tubing (length and flexibility)
- general suitability.

8.7.5.4: Checks to be performed by processing team

The processing team will follow routine procedures for recording pack faults, but may additionally wish to comment on:

- breakage rates following freezing
- heat seal failures (in-house seals)
- suitability of tubing (length and flexibility)
- ease of cannula breakage
- ability to sterile dock (during secondary processing)
- integrity of join, following local, current, procedure
- compatibility with instructions for device for sterile connection
- assess compatibility with current protocol for packaging of frozen packs
- inspection of packs after overnight storage at 4°C.

8.7.5.5: Component quality monitoring

A minimum of 1% of components (or as determined by statistical process monitoring) produced for whole blood collection processes or 300 of each component (one of each relevant component per procedure) for apheresis collection will be subjected to routine quality monitoring for parameters specified in this book. Deviation from the protocol must be consistent with section 8.7.1.

8.7.5.6: End users

Set up a process by which users will feedback information on acceptability of the packs for use. This would involve blood bank and ward/theatre staff. Obtain details on:
Blood bank issues:

- acceptability to end users
- acceptability of number and condition of bleed line samples
- crossmatch/other label adherence
- leak and breakage rates.

Ward/Theatre staff issues:

- general acceptability
- accessibility of ports for giving sets
- leak and breakage rates.

On completion, the individual or group responsible for the trial will prepare and submit a Phase 2 report on the suitability for use of the blood pack system within the service undertaking the trial.

8.8: Further guidance on the evaluation of blood packs and apheresis collection systems containing new plasticisers and additive solutions, where they are combined

8.8.1 Basic information about the Blood pack / Additive solution

The manufacturer must provide the following information for review:

- Instruction for use
- Information on composition and relative weights/volumes of the plastic / plasticiser of the bag and additive solution
- The minimum and maximum volume of whole blood or component that can be stored in the bag
- The shelf-life and storage conditions of incoming goods
- Evidence that the blood bag / additive solution or combination is CE/UKCA marked as a Medical Device
- Quantification of leaching of plasticisers into blood components. Manufacturers may work with Blood Services on these studies
- Studies on toxicology should be supplied by the manufacturer or be available from peer-reviewed scientific journal and should support the safety for use as a medical device. Where the toxicology data has been supplied by a manufacturer, there must be evidence of independent review by relevant external bodies with toxicology expertise.

8.8.2 Phase 0: Evaluation

This phase covers the initial assessment. Transfusion services may work alongside manufacturers to complete developmental work.

- Control data from routinely used blood bags should be included within the study
- The minimum number of units tested should be 16
- Due to the fact that plastics/plasticisers are under investigation, pool and split study designs may not be possible and where possible whole systems should be evaluated. Intermediate bags, tubing and needle assemblies will themselves contain plasticisers that could leach into components and thereby

influence results; this must be taken into account during study design. Advice from SACBC should be sought where required.

The following information should be recorded:

- Whole blood collection or Apheresis
- Conditions of overnight hold (if applicable)
- Collection volumes
- Mix of ABO groups (for plasma studies)
- Gender mix of donors
- Additive solutions used.

Data should be provided as close as possible to provide starting levels, mid-point and end of storage shelf-life measures being assessed. Plasma components should be sampled prior to freezing to allow assessment of the percentage recovery of coagulation factors. Further samples will be taken during storage and/or at the expiry of the component. This allows assessment of the impact of the novel system over the shelf-life of the components.

The phase 0 parameters to be validated are summarised in Tables 8.2, 8.3b and 8.4. The basis for acceptance criteria should be 'no worse than current' available systems, comparing against specifications where available and routine control data and where necessary published data. Advice from SACBC should be sought where required.

- Table 8.2 provides guidance on the specific tests recommended for assessing Red Cell components. For novel plasticisers, the tests specified for novel additive solutions are all relevant, with the additional parameter of '*Recovered plasticiser in supernatant and cells*'.
- Tables 8.3a and 8.3b provides guidance on the specific tests recommended for assessing platelet components. For novel plasticisers, the tests specified for novel additive solutions are all relevant, with the additional parameter of '*Recovered plasticiser in supernatant and cells*'. Plasma content and Plasma:PAS ratio are not required.
- Table 8.4 provides guidance on the specific tests recommended for assessing plasma components. For novel plasticisers, the tests specified for novel filter are all relevant, with the additional parameter of '*Recovered plasticiser in supernatant and cells*'.

For platelet components, there are three possibilities to consider:

- A new plasticiser for the whole blood system
- A new plasticiser for the platelet storage bag
- A new plasticiser for the apheresis collection set.

Where the whole blood system is novel, then the final platelet product derived from whole blood via buffy coat or PRP must be tested to demonstrate that it is similar (or no worse) to current platelet components. Some test parameters, for example '*Recovered plasticiser in supernatant and cells*' may have already been completed under red cell testing and this would not change for platelet storage.

For plasma components, there are three possibilities to consider:

- A new plasticiser for the whole blood system
- A new plasticiser for the plasma storage bag
- A new plasticiser for the apheresis collection set.

Where the whole blood system is novel, then the final plasma product derived from whole blood must be tested to demonstrate that it is similar (or no worse) to current product. Some test parameters, for example '*Recovered plasticiser in supernatant and cells*' may have already been completed under red cell testing and this would not change for plasma storage.

8.8.3 Plasticiser Analysis

Blood bag manufacturers or external laboratories may be required for chemical analysis of plasticisers. The chosen methodology will be specific to the plasticiser under investigation, but likely to be by liquid chromatography-mass spectrometry. It is important to have a validated analytical method for the detection of the plasticiser(s), with high sensitivity for the plasticiser and its major metabolites. Advice can be sought from manufacturers, SACBC and peer-reviewed literature. It is important to consider metabolites that may also influence product quality and may have toxicological effects. Concentrations in the supernatant (and cellular content for red cells and platelets) should be measured at the beginning, during and at end of storage to assess leaching and potential patient exposure. Consideration must be given to the effects of irradiation on the bag and subsequent leaching potential. Suppliers must undertake toxicology studies as part of CE/UKCA/UKNI marking. Suppliers must provide evidence of an independent review of toxicology data; this data will then be reviewed by SACBC.

8.8.4 Routine Process Evaluation

There is specific guidance on the evaluation required in section 8.7. Only specific points relevant to novel plasticisers are highlighted here:

- Due to the inclusion of a new plasticiser, label adhesion for base label and processing labels (donation number, component type, ABO or full face labels) should be confirmed
- The manufacturer should provide evidence to support the safety of glue and ink from base label migration across the bag as this migration may be affected by new plasticisers
- Collection and Processing teams should assess the pack assembly for general suitability and defects as per routine processes. If the needle assembly and tubing includes novel plasticisers, then special attention should be placed on suitability of use.

During phases 1 and 2, Processing teams should record processing faults, with special emphasis on:

- Breakage rates particularly following freezing, thawing and centrifugation
- Heat seal failures
- Flexibility of tubing and bag material with consideration for apheresis collection systems
- Transparency/opacity of tubing bags- this can affect sensors on automated separators
- Cannula breakage
- Ability to sterile dock: integrity of joint; compatibility with other tubing and docking machine
- Blast freezing and packaging of frozen components compatibility
- Irradiation of components: opacity/transparency; increased fragility.

8.8.5 Clinical Data

During phases 1 and 2, consideration should be given for the collection of clinical data on red cell, platelet and plasma components. This may include measures of haemostatic effect, *in vivo* recovery and survival and corrected count increment following transfusion.

Clinical studies/observations should include human data on transfusion reactions, other safety considerations, and efficacy. Clinical studies comparing blood components from existing and novel blood bag systems are required and advice can be sought from SACBC.

Observational data and post marketing surveillance data including national haemovigilance data must be available or in collection.

8.9: References

1. Murphy S, Rebulla P, Bertolini F, Holme S, Moroff G, Snyder E, Stromberg R (1994). In vitro assessment of the quality of stored platelet concentrates. The Biomedical Excellence for Safer Transfusion (BEST) Task Force of the International Society of Blood Transfusion. *Transfusion Medicine Review*, 8(1), 29–36.
2. Good Automated Manufacturing Practice (GAMP) Guide for *Validation of Automated Systems in Pharmaceutical Manufacture*. Available at www.ispe.org.